

AMENDED BRIEF DESCRIPTION OF THE FIGURES

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Fig. 1 **FIGURE 1** shows the hypersensitivity of gyrase mutant alleles in terms of the fold increase in sensitivity toward 12 antibacterial agents and generally toxic agent for 3 three temperature sensitive mutants of *Salmonella typhimurium*. These are mutants of DNA gyrase subunit A (gyrA212, gyrA215, and ~~gyrA216~~ gyrA216), grown at a semi-permissive temperature (~~35-6~~ 35 °C). Hypersensitivity is observed to antibacterial agents acting on DNA gyrase, but not to other classes of drugs or toxic agents. The data demonstrate that growth conditional mutations in a known target cause hypersensitivity to target inhibitors.

Fig. 2 **FIGURE 2** presents the hypersensitivity profiles of a set of temperature sensitive mutants of *Salmonella* for a variety of antibacterial agents with characterized modes of action compared to the sensitivity profile of wild type. The following abbreviations are used in the Figure: Nov - novobiocin; Cou - coumermycin; Cip - ciprofloxacin; Nor: norfloxacin; MitoC: mitomycin C; ΦHg: phenylmercuric acetate; NQO: 4-nitroquinoline oxide; Rif: rifampicin; Gen: gentamicin; Strep: streptomycin; Phen: phenol; Cefo: cefotaxime; Amp: ampicillin; Fosfo: fosfomycin; clm?: unknown conditional lethal mutant; round: round cell morphology; Thy inc-: defective thymidine incorporation phenotype; Odd: odd cell shape morphology; Filam: filamentous cell morphology; UV-: ultraviolet sensitivity. Known or closely related genotypes: *dnaE*: DNA polymerase III α subunit; *gyrA*: gyrase subunit A; *parC*: *gyrA*-like subunit of topoisomerase IV; *parE*: *gyrB*-like subunit of topoisomerase IV; *parF*: acetyl transferase activity associated with the topoisomerase IV gene; *murB*: UDP-N-acetylglucosaminyl-3-enolpyruvate reductase; *dapA*: dihydrodipicolinate synthase; *murCEFG*: *nea4* cluster of L-Ala, DAP, D-Ala-D-Ala and NAG ligase; and, *fts-H*: may be *ftsH* by map location.

Fig. 3 **FIGURE 3** illustrates a variety of types of interactions which exist between different essential genes and which can create differential responses in screens using growth conditional mutants.

Fig. 4 **FIGURE 4** illustrates a possible arrangement of a multi-channel screen plate using conditional growth mutants with mutations affecting 5 different cellular processes plus controls.

Fig. 5 **FIGURE 5** illustrates operational designs for a multi-channel screens. Two alternative multi-screen designs are shown in which either multiple compounds are screened using a single growth conditional mutant on each plate, or in which multiple growth conditional mutants are used on each plate to create an inhibition profile of a single compound.

Fig. 6 **FIGURE 6** is a bar graph showing the different heat sensitivity profiles for ~~[[4]]~~ four different *S. Aureus* heat sensitive mutant strains. The growth of he ach strain is shown at 6 different temperatures ranging from 30 °C to 43 °C.

Fig. 7 **FIGURE 7** is a bar graph showing the different heat sensitivity profiles for ~~[[4]]~~ four different *S. aureus polC* heat sensitive mutants and a wild type strain. The growth of each strain is shown at 6 different temperatures ranging from 30 °C to 42 °C.

Fig. 8 **FIGURE 8** is a graph showing the difference in hypersensitivity of one *S. aureus* heat sensitive strain (NT99) toward 30 inhibitory compounds at ~~[[3]]~~ three different temperatures.

Fig. 9 **FIGURE 9** is a diagram for two *S. aureus* mutants, illustrating that a greater number of growth inhibitory hits are identified at higher temperatures using heat sensitive mutants. Compounds were identified as hits if the growth of the mutant was inhibited by at least 50% and the inhibition of growth of the mutant was at least 30% higher than the inhibition of growth of a wild type strain.

Fig. 10 **FIGURE 10** is a bar diagram illustrating the effect of test compound concentration on a number of hits identified, showing that, in general, more compounds are identified as hits at higher concentrations.

Fig. 11 **FIGURE 11** presents the structures of two compounds which exhibited ~~the same~~ similar inhibition profiles for a set of temperature sensitive ~~Staphylococcus~~ *S. aureus* mutants, showing the structural similarity of the compounds.

Fig. 12 **FIGURES 12A AND 12B** presents the ~~fold-increase~~ fold-increase in sensitivity of a set of ~~Staphylococcus~~ *S. aureus* temperature sensitive mutants for a

variety of compounds which inhibit growth of *Staphylococcus S. aureus* wild type, but which have uncharacterized targets of action.

Fig. 13 **FIGURE 13** illustrates the types of anticipated inhibition profiles of different growth conditional mutants for a variety of test compounds, indicating that the number of mutants affected by a particular compounds is expected to vary.

Fig. 14 **FIGURE 14** shows the proportion of compounds (from a total of 65) which significantly inhibited the growth of varying numbers of temperature sensitive mutants in a screen of uncharacterized growth inhibitors of *Staphylococcus S. aureus*.

Fig. 15 **FIGURE 15** shows the potency (MIC values) of a number of growth inhibitors which affected 0, 1 or more than 3 temperature sensitive mutants of *Staphylococcus S. aureus* in a screen of 65 uncharacterized growth inhibitors.

Fig. 16 **FIGURE 16** shows the number of hits for each of the temperature sensitive mutants of *Staphylococcus S. aureus* in a screen of 65 uncharacterized growth inhibitors.

Fig. 17 **FIGURE 17** shows some advantages of a ~~multichannel~~ multi-channel genetic potentiation screen using growth conditions mutants over traditional biochemical screens with either a known target or an unknown cloned gene.

Fig. 18 **FIGURE 18** illustrates a strategy for selecting dominant lethal mutants for use in screens for antibacterial agents[[,]] not requiring hypersensitivity.

Fig. 19A-D **FIGURES 19A - 19D** ~~are show the structures for of~~ show the structures for of four compounds which ~~that~~ were identified as hits ~~on using~~ using mutant NT94.

Fig. 20 **FIGURE 20** is a partial restriction map of the *S. aureus* clone insert (complementing mutant NT64)[[,]] showing the position of the initial left and right sequences obtained.

Figs. 21-90 **FIGURES 21 - 90** are partial restriction maps of each of the *S. aureus* clone inserts for which sequences are described herein[[,]] showing the relative fraction of the insert for which a nucleotide sequence is described[[,]] as well as the approximate position of identified open reading frames (ORFs).